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Recommended Citation

Ashby, Alison Aria, "The Effects of Stocking Density, Amount of Substrate, Frequency of Feeding, and Waste Removal on Nursery Production and the Effects of Substrate Height on Pond Production of Freshwater Prawn *Macrobrachium rosenbergii*. " Master's Thesis, University of Tennessee, 2003.
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I am submitting herewith a thesis written by Alison Aria Ashby entitled "The Effects of Stocking Density, Amount of Substrate, Frequency of Feeding, and Waste Removal on Nursery Production and the Effects of Substrate Height on Pond Production of Freshwater Prawn *Macrobrachium rosenbergii*." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

Dr. J. Larry Wilson, Major Professor

We have read this thesis and recommend its acceptance:

Dr. Thomas K. Hill, Dr. Barry Sims

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Alison Aria Ashby
December 2003

ACKNOWLEDGEMENTS

I am indebted to Dr. Larry Wilson for giving me the opportunity to pursue an advanced degree in fisheries, and for providing the guidance and encouragement needed to complete this study. Special thanks to the rest of my committee, Dr. Thomas Hill and Dr. Barry Sims, for their assistance and support throughout the duration of this study. Great appreciation is expressed to Dr. John Taylor for the guidance and support he offered me throughout this effort. His continued encouragement and assistance were vital to the completion of this study.

I also thank my fellow graduate students, Joyce Coombs, Kimberly Cooper, Virginia Harrison, Justin Laughlin, and Todd Sink for their assistance with prawn counts and feedings. Also, thank you to the Spring Camp students, Matt Troxler, and my father for helping with data collection. I also want to extend appreciation to the University of Tennessee staff at the Joseph E. Johnson Animal Research and Teaching Unit for their technical assistance throughout the project. I express thanks to Bill Pitt and the staff at the Highland Rim Experiment Station for managing the ponds for the experiment.

I am very grateful for the funding from the Department of Forestry, Wildlife and Fisheries which was provided in the form of a tuition waiver and stipend for a teaching assistantship. Special thanks to Dr. Scott Schlarbaum for his financial assistance and support throughout my graduate career. Finally, I would like to thank my husband, Avery Ashby, for his tireless support and encouragement, and for his statistical insight.

ABSTRACT

Production of freshwater prawn (*Macrobrachium rosenbergii*) in temperate regions has gradually increased over the past decade due to advances in research. However, production is limited to one seasonal crop in these regions, increasing the need to intensify production. A nursery phase has been implemented to produce larger juveniles to stock into production ponds.

Three nursery experiments (A, B, C) were performed to evaluate the effects of stocking density, amount of added substrate, feeding frequency, and waste removal on the survival and growth of juvenile prawn. Experiment A involved two concurrent studies. Prawn averaging 0.01 g were stocked at densities of 2.5, 5, 7.5, and 10/L into 24 200-L tanks. The surface area of each tank was 15,485 cm^2; horizontal layers of polyester mesh (6 x 6 mm) were added to equal 50 or 100% increase in tank surface area. The first study compared the two amounts of substrate at two densities (5 and 7.5/L). The second study compared four different densities (2.5, 5, 7.5, and 10/L) with the smaller amount of substrate. Each treatment combination of stocking density and substrate amount was replicated four times. After 60 days, the first study revealed that prawn stocked at 5/L had significantly higher survival (73 and 64% for 5 and 7.5/L, respectively), but lower average individual weight (0.13 and 0.14 g for 5 and 7.5/L, respectively) than those stocked at 7.5/L. Substrate equal to 100% of the tank's surface area did not significantly increase survival over tanks with 50% added substrate, but did produce significantly larger prawn (0.15 vs. 0.12 g). In the second study, the only significant difference in survival was with prawn stocked at 5/L (72%) versus 2.5/L

(55%). Prawn stocked at 2.5/L had an average individual weight (0.08 g) that was significantly less than prawn stocked at 5 (0.12 g), 7.5 (0.13 g), or 10/L (0.13 g).

Experiments B and C were initiated with 0.016 g postlarvae stocked at 5/L in circular and rectangular 400-L tanks. In the six circular tanks, polyester mesh (6 x 6 mm) was added in horizontal layers to increase the surface area of the tank by 53% (46.454 cm^2). Prawn were fed either a single daily ration or two daily rations that were each equal to half of the single ration, according to a percent body weight regimen. After 60 days, there were no significant differences in survival or average individual weight of prawn fed once daily (66%, 0.13 g) versus twice daily (55%, 0.13 g). In the rectangular tanks, horizontal layers of polyester mesh were added to increase each tank's surface area by 45% (46,454 cm^2). Three tanks were siphoned three times weekly to remove uneaten feed; the three other tanks were never siphoned and uneaten feed and wastes were allowed to accumulate. After 60 days, there were no significant differences in survival or average individual weight of prawn in tanks where waste was removed (57%, 0.11 g) versus tanks where waste was allowed to accumulate (67%, 0.14 g).

One pond experiment was conducted to examine the effects of substrate height on production. In the pond experiment, prawn averaging 0.14 g were stocked at a density of 53,304/ha in six 0.04-ha ponds. Three ponds had 0.61-m high fencing and three had 1.22-m fencing, increasing surface area by 20 and 40%, respectively. After 106 days, no significant differences were found in prawn survival (70 vs. 85%), yield (1296 vs. 1508 kg), mean individual weight (32.2 vs. 29.9 g), or feed conversion (2.14 vs. 1.84) for ponds with 0.61-m high versus 1.22-m high substrate, respectively.

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CHAPTER I

INTRODUCTION

The giant Malaysian prawn (*Macrobrachium rosenbergii*) has received special interest in the southeastern United States as a prospective culture species because of its rapid growth rate, large maximum size, relative hardiness, adaptability to culture conditions, and potential consumer demand. *M. rosenbergii* is native to the tropical Indo-Pacific region. Early culture of freshwater prawn occurred during the 1950's in Malaysia where they were reared in captivity, either through introducing wild-caught juveniles or by trapping them with other crustaceans (Wickins 1976). In Thailand during the 1950's, prawn postlarvae were caught in their natural habitat and transported to artificial ponds for grow-out (Sandifer and Smith 1985). Experiments on the rearing of prawn larvae had been unsuccessful until 1961 when Shao-Wen Ling discovered that freshwater prawn larvae require brackish conditions for survival (New and Valenti 2000).

The commercial development of freshwater prawn was made possible when broodstock of *M. rosenbergii* were introduced from Malaysia into Hawaii (Fujimura and Okamoto 1972). During the 1970's, due to the availability of postlarvae for stocking, experiments were conducted in Hawaii and a significant industry began to develop in Thailand and Taiwan. The success of the hatchery and grow-out experiments conducted in Hawaii encouraged the introduction of broodstock into many countries where *M. rosenbergii* was not indigenous including North, Central, and South America, Africa, and even Europe for experimental environmentally controlled culture (New and Valenti 2000).

In the continental United States during the 1970's and 1980's, research related to hatchery, nursery, and pond production techniques, as well as marketing, were conducted in Florida, Georgia, Kentucky, Louisiana, Mississippi, South Carolina, and Texas (Smith et al. 1976; Wickens 1976; Sandifer and Smith 1977; Smith and Sandifer 1979a; Smith and Sandifer 1979b; Smith et al. 1981; Smith et al. 1983). Prior to the early 1990's, it appeared that culture of the freshwater prawn was not economically feasible (D'Abramo et al. 2002). However, due to the extensive research programs developed at Mississippi State University and Kentucky State University, new management practices and production techniques have increased the potential for economic success. As a result, the freshwater prawn industry has begun to develop across the southern United States and in Tennessee during the past three years. It is estimated that in 2002, approximately 200 acres were utilized to farm freshwater prawn in Tennessee (Thomas K. Hill, personal communication).

This study involved three different nursery trials and one pond grow-out experiment. It was designed: (1) to evaluate the effects of four different stocking densities and two different substrate quantities on juvenile prawn survival and growth; (2) to evaluate survival and growth of prawn fed once versus twice per day; (3) to evaluate the effects of waste removal on survival and growth of prawn; and (4) to test the effects of two different heights of substrate on the survival and growth of prawn reared in ponds.

CHAPTER II

LITERATURE REVIEW

Macrobrachium is the largest genus of the family Palaemonoidea. This genus is circumtropical and native to all continents except Europe (New and Valenti 2000). Approximately 200 species have been described in this genus, with all living at least part of their life in freshwater. Several species of *Macrobrachium* are indigenous to the southern United States, but none have commercial potential because of their small maximum size.

M. rosenbergii is easily distinguished from other species of the genus by the combination of several characteristics. It is the largest of all *Macrobrachium* species with a total body length up to 320 mm. It has a long rostrum with 11 to 14 dorsal teeth and 8 to 10 ventral teeth. The adult male has very long second chelipeds in which all segments are elongate and provided with a blunt spine, and the movable finger of the second chelipeds is covered by a dense velvet-like fur in the adult male (New and Valenti 2000).

M. rosenbergii live in tropical freshwater environments which are often turbid. They have a hard outer skeleton which is shed regularly during periods of growth. Females are commonly reproductively mature before six months of age. Mating only occurs between soft-shelled females or females who have recently molted and hard-shelled males. Females carry eggs in a gelatinous mass on the underside of the abdominal region between the fourth pair of walking legs. They move downstream into brackish estuaries where eggs hatch as free-swimming larvae. Larvae undergo 11 distinct zoel stages over 15 to 40 days before becoming postlarvae (PL). The prawn go from swimming upside down and backwards in the water column during larval stages, to a more benthic lifestyle as they begin to migrate upstream towards freshwater. After metamorphosis to postlarvae takes place, they resemble adult prawn.

M. rosenbergii is an omnivorous animal that feeds on a large array of items in its natural environment. As larvae, the freshwater prawn is an aggressive sight feeder that feeds primarily on zooplankton, worms, and larval stages of aquatic insects. The diet of postlarvae includes larval and adult insects, worms, algae, mollusks, crustaceans, feces of fish and other animals, and plant material. Prawn become cannibalistic when stocked at high densities or during periods of limited food availability.

Freshwater prawn are tropical crustaceans, and are sensitive to cold. Optimum temperatures for growth are approximately 28 to 31°C (New and Valenti 2000). In temperate regions, production involves three distinct phases: brackishwater larval period (hatchery); freshwater indoor-tank period of growth to advanced stocking size (nursery); and pond grow-out (D'Abramo et al. 2002). The nursery phase supplements the limited growing season (120 days) in temperate climates by growing post-larval prawn to advanced juvenile stages in temperature-controlled systems prior to pond stocking. Nurseries permit the production of larger juveniles for stocking into ponds. The stocking of larger juveniles results in increased production, higher crop value, and improved overall success rates. Many investigations have been conducted to maximize growth and survival in order to provide a cost effective nursery phase for the growth of *M. rosenbergii* postlarvae. Studies related to stocking density, added substrate, feeding rates and schedules, and water quality of both nursery and pond grow-out phases of prawn culture have provided valuable information important in achieving maximum survival and growth rates.

Several studies have been conducted to evaluate survival and growth rates of prawn stocked at different densities during the nursery phase. Twelve nursery trials were conducted in two phases by Smith et al. (1983). In phase I, prawn with an average individual weight of 0.1g, were stocked at densities of 1194-6276/m^2 tank bottom for 67-105 days. Survival was comparable after 4, 8, and 12 weeks, averaging 96, 93, and 90%, respectively, and prawn had a final weight of 0.4 g. In phase II, prawn with an average individual weight of 0.7-0.9 g, were stocked at densities of 538-904/m^2. Survival for 4 and 8 weeks averaged 91 and 84%, respectively, and prawn attained a weight of 0.9-2.1 g.

Marques et al. (2000) evaluated the effects of stocking density on growth, biomass increase, and survival of postlarvae stocked in cages. The study involved two phases where newly metamorphosed postlarvae (PL) were stocked for 20 days at densities of 2, 4, 6, 8, and 10/L. In the second phase, postlarvae were stocked for 60 days at 100, 200, 300, 400, 600, and 800/m^2. In the primary phase, survival, final mean weight, and average weight gain were significantly lower at higher densities and biomass was significantly higher. In the secondary phase, final mean weight and average weight gain were significantly higher for the lowest two densities and biomass was significantly higher at densities of 400, 600, and 800 PL/m^2. There were no significant differences in survival among the densities.

Smith and Sandifer (1979a) conducted a series of eight nursery trials to evaluate population densities of 300, 1000, and 1500 prawn/m^2 tank bottom area. They observed that growth was similar for all densities for the first month, after which prawn size increased in the lowest density. During the first two months of the study, growth was similar at densities of 1,000 and 1,500. After 60 days survival rates were similar at 91, 91, 82% for densities of 300, 1000, and 1500, respectively. Kneale and Wang (1979) also evaluated the effect of stocking density on survival and growth. In their study, survival rates were higher (84%) at lower stocking densities of 150 PL/m^2 compared to higher densities of 1500 PL/m^2 (39%). Growth was greatest for stocking densities at or below 600 PL/m^2.

Environmental conditions, such as water quality and nutrition, are factors which affect postlarvae physiological state and, in turn, survival and growth rates. An experiment conducted by D'Abramo et al. (2000) evaluated the effects of combinations of water volume, bottom surface area, and water replacement rate on weight gain of juvenile prawn. Prawn were reared in a flow-through system, with each tank receiving an independent water supply. After 60 days, weight gain of prawn was significantly higher under conditions of greater water volume and bottom surface area and under conditions of a greater bottom surface area with lower water replacement rates. A similar study conducted on white shrimp (*Litopenaeus setiferus*) found that the optimum shrimp density-water exchange combination was between 5 to 12% water exchange per day at a stocking density between 50 and 150 shrimp/m^2 (Palomino et al. 2001).

Prawn nutrition has long been a topic of experimental investigation; however, little is know about feeding schedules. Heinen and Mensi (1991) conducted an experiment in which prawn were fed once daily (at 1600 h), twice daily (at 0800 and 1600 h), twice daily (at 0800 and 2000 h), and three times daily (at 0800, 1600, and 2400 h). Prawn fed once per day exhibited the highest survival, final weight, yield, and food conversion ratio.

The amount of surface area available in culture tanks and ponds is often a limiting factor leading to reduced survival and growth rates. Due to the cannibalistic nature of prawn, Ling (1969) recommended that aquatic plants and branches be used in ponds to provide refuge from aggressive encounters. This recommendation has also been used in nursery systems. Studies have proven that artificial substrates increase survival in nursery systems and ponds, while allowing for an increase in stocking density. Smith and Sandifer (1975) compared horizontal and vertical substrate versus no added substrate. After four weeks, prawn mortality was four times greater in tanks not containing substrate than in tanks fitted with five-tiered horizontal substrate units. Survival was slightly higher among prawn reared in tanks containing horizontal substrate (65.3%) compared to vertical substrate (54.5%). Marcus (2002) found that there was no significant difference in survival or average individual weight of prawn reared in tanks with horizontal substrate (56%, 0.34 g) compared to vertical substrate (53%, 0.36 g) after 65 days. In the same study, the effects of substrate color, tan or brown, on survival and growth were compared. There were significant differences in survival rates between tan (40%) and brown (46%) colored substrates, but no difference in growth was found. Molina (1990) also compared substrate color, black and white habitats, to the lack of substrate. No significant differences in mean survival (76%), average weight gain (0.55 g), total feed conversion ratios, or yield were found among prawn with black habitat substrate of different mesh sizes and material. A white nylon 1.27-cm mesh habitat produced significantly greater survival (83.5%). Tanks that lacked substrate had significantly lower survival (33%), but higher mean weight gain (0.75 g). The Molina (1990) study found prawn stocked at 7/L exhibited higher survival and growth than prawn stocked at 9 or 11/L.

An investigation conducted by Sandifer and Smith (1977) found that tank bottom substrate did not significantly affect prawn growth or survival. They compared three replicate tanks which contained in-tank dolomitic gravel filter beds to tanks with bare floors. Two similar studies compared tank floors covered with sand, gravel, or pond soil to bare tank floors. Smith and Sandifer (1979a, 1979b) monitored the behavioral responses of prawn to different habitat configurations, types, and materials. Layered habitats were found to be well utilized by prawn. Prawn tended to concentrate at the edges of habitat materials, with 70-80% of prawn located near the edges. A layered mesh habitat consisting of alternating open and strip layers was recommended to enhance utilization and create a homogeneous distribution within the habitats. Large mesh plastic screening (3.8 cm^2) was tested against fiberglass strip layers to show that postlarvae had a significant preference for the mesh. Larger prawn were observed in the lower habitat levels and areas directly beneath the habitat.

Similar studies have been conducted during the pond grow-out phase of culture to evaluate the effects of stocking density, added substrate, population structure, and harvesting methods on prawn production. An investigation performed by Tidwell et al. (1998) found that prawn raised in ponds containing added substrate had growth rates and population structures characteristic of prawn stocked at lower densities. In their study, prawn were stocked at a density of 59,280/ha into six 0.04-ha ponds. Three ponds contained added substrate consisting of PVC frames with horizontal plastic mesh and vertically suspended seines which increased available surface area approximately 20%; three control ponds received no added substrate. No significant differences in survival were found between treatments; however, average individual weights of blue claw males, orange claw males, reproductive females, and virgin females were significantly higher in ponds with added substrate (37 g) compared to ponds without substrate (30 g).

Tidwell et al. (2002) also tested the effects of substrate, but went a step further and investigated substrate orientation and amount. Their study compared the effectiveness of polyethylene fencing oriented horizontally or vertically, and evaluated increasing amounts of vertical substrate (50 and 100%). After 106 days, substrate orientation did not have a significant impact on prawn survival, production, or population structure. However, total yield was greater in ponds with 100% increase in surface area (2,653 kg/ha) than in ponds without substrate (2,452 kg/ha). In a similar study, added substrate produced a significant increase in production (18%) (Tidwell et al. 1999). Plastic mesh and strips of oyster netting were oriented horizontally in ponds to increase surface area by 80%. Prawn were stocked in 0.04-ha ponds at densities of 60,000 and 120,000/ha with and without substrate. Prawn stocked at high densities had significantly greater production (1,587 kg/ha) than prawn stocked at low densities (1,156 kg/ha); however, average individual weight decreased in prawn stocked at high densities.

Size grading nursery populations before stocking into production ponds has been found to be beneficial by increasing individual mean harvest weight and total yield. Size grading disrupts the social hierarchy among males by separating the faster growing prawn from the slower. Separating the size classes also allows smaller males to increase growth rates to compensate for initial slow growth rates, which results in a reduction in the range of sizes at harvest. D'Abramo et al. (2002) recommended that once prawn weight is determined, a 50%-50% (upper-lower) or 40%-60% numerical separation should be used so that the entire population can be used for stocking.

Daniels et al. (1995) stocked size-graded (0.25 ± 0.086 g) juvenile prawn at densities of 39,540, 59,300 and 79,100/ha for 131 to 134 days in three earthen ponds. Prawn in these ponds were fed a specially formulated 34% protein prawn diet. Three additional ponds were stocked at 39,540/ha and fed a commercially available sinking catfish feed. There was a significant difference in mean wet weight of prawn stocked at 39,540/ha (34.3 g) compared to those stocked at 59,300/ha (26.7 g) and 79,100/ha (26.3 g). Feed type did not have a significant effect on growth.

Smith et al. (1981) examined various stocking strategies and densities in ponds. The strategies tested consisted of stocking postlarvae only, a mixed population of postlarvae and juveniles, and juveniles only at densities from 2.15-8.61 prawn/m^2 (21,500-86,100/ha). Stocking postlarvae resulted in low mean survival rates (69.6%) compared to stocking juveniles or a mixed population. Stocking juveniles resulted in increased size at harvest (22.1 g); however, stocking a mixed population of postlarvae and juveniles at a density of 6.5/m^2 produced similar results (23.1 g). Mean survival rates for stocking juveniles only or a mixed population of postlarvae and juveniles were 81 and 73%, respectively.

Smith et al. (1976) evaluated the population structure of prawn at harvest. They found that initial prawn size at stocking was the most important factor controlling size distribution. Stocking juveniles resulted in larger size classes at harvest. In contrast, the stocking of postlarvae resulted in 46 to 96% of the harvested biomass being in the small

(<15.0 g) size classes compared to stocking juveniles. In general, they found that sex ratios were biased toward females, with the total biomass of females being 20.6% greater than males. Mean weight of males and females at harvest was found to be similar, although males exhibited much greater variation in size. Similarly, Siddiqui et al. (1995) found that females (67.4%) were more abundant than males (32.6%) in a study that examined the effects of harvesting methods (cull and batch) on population structure, growth, and yield of prawn. Prawn were stocked at two different densities (5 and $10/m^2$) in eight tanks. Four tanks were randomly selected for cull harvesting at 28-day intervals at which time larger prawn were harvested, and the remaining four tanks were harvested at the end of the experiment (196 days). There were no significant effects of harvesting methods or stocking densities on sex ratio. Mean survival was highest (92.6%) at a stocking density of $10/m^2$ and when prawn were cull harvested; however, mean weights ranged from 25.3 to 29.9 g and were not significantly different from one another.

Many nursery and pond grow-out experiments involve sampling prawn at various stages throughout the study, which increases handling. It has been suggested that experimental practices which involve test animals being subjected to repeated handling are often more invasive than those used by commercial growers. Frequent handling may lead to increased stress levels directly, but may also lead to antagonistic interactions when the animal is returned to the water and has to reestablish a social hierarchy. Relatively no research has been conducted on the effects of increased handling on prawn survival and growth, but several articles on marine and freshwater crayfish (Davis 1981; Brown and Caputi 1985; Morris and Callaghan 1998; Evans et al. 1999) have suggested that handling may result in stress or retardation of growth.

Farrell and Leonard (2000) examined the effects of frequent handling on the Australian freshwater crayfish (*Cherax destructor*). They found that frequent handling was significantly and negatively correlated with weight gain. They hypothesized that the process of sampling increased the frequency of behavioral interaction, which in turn affected growth by stressing the animals. Another factor that may explain the linkage between handling and low growth was the exposure of the crayfish to air during sampling.

CHAPTER III

METHODS

Three separate nursery experiments (A, B, C) with freshwater prawn postlarvae, *Macrobrachium rosenbergii*, were conducted sequentially from April 2002 to June 2002 at the Joseph E. Johnson Animal Research and Teaching Unit, University of Tennessee, Knoxville. One pond grow-out experiment was conducted at the University of Tennessee Highland Rim Experiment Station, Springfield, Tennessee, from 6 June 2002 to 25 September 2002.

Nursery Operations

Experiments A, B, and C were each conducted over a duration of 60 days. Experiment A assessed the effects of four different stocking densities (2.5, 5, 7.5, and 10/L) and two quantities of substrate (50 and 100% increase in surface area) on survival and mean average weight of prawn. Experiment B evaluated survival and growth of prawn fed once versus two times per day. Experiment C was conducted to test growth and survival of prawn in culture tanks where waste was removed regularly versus not removed for the duration of the trial.

Prawn were obtained from Craig Upstrom at Aquaculture of Texas (Weatherford, Texas) for Experiment A and from Steve and Dolores Fratesi at Lauren Farms, Inc. (Leland, Mississippi) for Experiments B and C. Prawn for Experiment A were shipped via air freight in eight plastic bags stocked at a density of 5g/L, with each bag containing 5000 prawn. Prawn for Experiments B and C were shipped via ground transportation in six plastic bags stocked at a density of 5g/L with each bag containing approximately 5000 prawn. The prawn for both shipping methods were packed in brackish water (5 ppt) to reduce ammonia-related stress and water temperatures were lowered to 22.2°C to reduce metabolic activity. Bags were filled 2/3 with pure oxygen and packed in insulated shipping boxes.

Culture System Design

Nursery experiments used two similar flow-through aquaculture systems. Experiment A utilized one system, and Experiments B and C were conducted simultaneously in the second system. Flow-through systems were used to minimize stress effects related to deterioration in water quality. In each system, municipal water was dechlorinated with a charcoal filter and routed through two carbon filters into a head tank. A valve was placed between the two carbon filters to allow for water quality monitoring. Water was routed through the filters to the two head tanks using 2-cm Tygon tubing. The head tanks were 1135.5-L Rubbermaid® containers, and each contained a float valve to control water level. They were positioned on a metal stand approximately 3 m off the floor and 0.5 m above the culture tanks to facilitate gravity flow.

Water temperatures were maintained in the head tanks at 25±0.5 °C by using two 1800-watt digitally controlled immersion heaters (Process Technology). An airstone was placed in each of the head tanks for water circulation and to help maintain a uniform temperature. A linear magnetic piston air pump (#MAPXL-MANAIR XL, Ultralife Reef Products) was used to distribute air to the culture tanks. Both water and airlines were constructed of polyvinyl chloride pipe (PVC). Water lines were 2 cm and airlines were

1.3 cm in diameter. Flow-through rates in the culture tanks were approximately 0.2L/minute, resulting in a 100% water exchange approximately every 16.5 hours in Experiment A and every 33 hours in Experiments B and C. PVC caps, with a hole drilled in the center of each, were used to regulate water inflow into each culture tank.

Temperature was maintained at 25.5± 0.5 °C and 24 hours of light was sustained in each room. Tidwell et al. (2001) found that postlarval prawn raised under continual light conditions had significantly greater survival (72%) than those raised under continual darkness (58%) or 12 hours light: 12 hours dark (59%). The flow-through systems were indoors and illumination was provided by fluorescent lights located on the ceiling. The nursery that contained Experiment A had two 1 x 1 m windows located on the northeast wall behind the head tank. The nursery that contained Experiments B and C had no windows and only received artificial light.

Each culture tank contained substrate to increase surface area. Substrate frames were constructed in a modified H-design by using 1.3-cm PVC pipes, four PVC elbows, and two PVC tees (Figure 1). Each frame had four legs with 1.3-cm PVC caps on the bottom of each. Holes drilled in the PVC legs attached the mesh with plastic zip ties to the frames and allowed the substrate to sink. A 6 x 6-mm polyester brown mesh was suspended in layers and attached to the frames.

Prawn in all three experiments were fed a commercially available pelleted feed formulated for penaeid shrimp (Rangen, Inc., #1) which consisted of 45% crude protein, 9% crude fat, 4% crude fiber, 15% ash, and 10% phosphorus. Prawn were fed 10% of their body weight on days 1-30, 8% on days 31-45, and 7% on days 46-60. Feed was

Figure 1. Substrate used for nursery culture.

stored in original bags sealed in plastic containers at 5°C in a walk-in cooler. Excess waste was removed from tanks using a siphon at determined intervals. A siphon was built using 2-cm Tygon tubing and a 2-cm PVC pipe, with 0.5-mm metal mesh covering the opening to prevent prawn from leaving the culture tanks. Water temperature was recorded daily to the nearest 0.1°C using a mercury thermometer. Water entering the head tank was also tested daily for chlorine using DPD free chlorine reagent powder pillows (Hach Permachem Reagents).

Data Collection

Prawn were counted and weighed at regular intervals throughout the duration of the three experiments. Prawn in Experiment A were counted and weighed at 30, 45, and 60 days. Prawn in Experiments B and C were counted at 60 days. On sampling days, each culture tank was individually drained to approximately 1/10 original volume using a siphon. Prawn were netted into plastic containers and individually counted. Groups of

100 prawn were placed into a dipnet with a narrow opening and a 1.3-cm diameter PVC pipe on the end. This allowed the net to be spun around and any excess water removed from the prawn. Prawn were weighed in groups of 100 on a digital Mettler scale (Denver Instrument Co.) and mean weights were determined. Prawn were then returned in groups of 300 to their original tanks. At the end of each count, survival rates, mean individual weight, and new feed rates were calculated.

Nursery Experiments

Experiment A

This experiment was conducted from 4 April 2002 to 3 June 2002 to assess the effects of four different stocking densities (2.5, 5, 7.5, and 10/L) and two different quantities of substrate (50 and 100% increase in surface area) on survival and mean average weight of prawn. Twenty-four oval tanks (200 L) were used for this experiment. The culture tanks were 91.4 cm long and 45.7 cm wide and contained 15,485 cm^2 of surface area. Each tank had a vertically positioned PVC standpipe 3.2 cm in diameter and 35.6 cm high connected to a 3.2-cm PVC elbow that directed overflow to a drain in the floor. The top of each standpipe was covered with a 0.5-mm metal mesh to prevent loss of prawn. The tanks were 45.7 cm in height, but only contained 35.6 cm of water to provide enough free-board to help prevent prawn from jumping out of the tanks. The tanks were set on a concrete floor.

Two separate water and airlines flowed from the head tank to two identical sets of 12 culture tanks. Treatments were randomly assigned to each of the two sets of culture tanks according to a 2 x 1 randomized complete block experimental design. The 5 and

7.5/L densities were the only treatments that had both the small and large substrate. Each treatment had four replications.

Substrate was positioned horizontally and used in all 24 tanks. The small substrate frames were 33 cm high, 50.8 cm long, and 35.6 cm wide. The frames contained four 30.5 x 45.7-cm layers of mesh suspended 8.9 cm apart. The smaller substrate amount, measuring the horizontal surfaces only, increased the total surface area of the tanks by 7,742.5 cm^2 (50%). The larger substrate frames increased surface area by 100% or 15,485 cm^2 and were 33 cm high, 75 cm long, and 42.5 cm wide; they contained four 39.4 x 71.1-cm layers of mesh suspended 10.8 cm apart.

Prawn, averaging 0.01 g, were stocked accordingly into each of the treatments. A total of 30,000 postlarval prawn were stocked randomly in groups of 500 into the culture tanks. Dead prawn were replaced after the second day of the experiment; approximately 1.3% of all stocked prawn were replaced. Prawn were fed twice daily at 0830 and 1800 hours and tanks were siphoned every three days to remove excess waste.

Experiment B

This experiment was conducted from 12 April 2002 to 11 June 2002 to evaluate survival and growth of prawn fed once versus two times daily. Six (400L) circular fiberglass tanks 121.9 cm in diameter and 49.5 cm in height were used for this experiment; each culture tank had 24,807 cm^2 of surface area with a vertically positioned PVC standpipe 6.4 cm in diameter and 55.9 cm high. The standpipe was attached to a 6.4- cm diameter PVC pipe, which connected each tank and directed water overflow to a drain in the floor. The top of each standpipe was covered with a 0.5-mm metal mesh to prevent spillover of prawn. Each tank was supported by four legs 11.4 cm in diameter and 12.7 cm high. The six circular tanks had water and airlines separate from the other culture tanks located in the same nursery.

Each tank contained four horizontally positioned substrate frames. Each frame was 46 cm tall, 50 cm long, and 33 cm wide. The frames contained four 31 x 36-cm layers of mesh suspended 6.0 cm apart from each other. The added substrate, measuring the horizontal surfaces only, increased the overall tank surface area to 46,454 cm^2 (87%).

Treatments were assigned in a random systematic format, with each treatment having three replications. Prawn were fed a daily ration based on percent body weight either once (entire amount) or twice (half single daily ration each feeding) daily. Prawn were fed daily at 0830 and 1800 hours, respectively. Prawn were individually counted and distributed randomly in groups of 500 into each of the six culture tanks. A total of 12,000 prawn with an average individual weight of 0.016 g were stocked at a density of 5/L (N=2000) into each of the six tanks. Tanks were siphoned every three days to remove uneaten feed and excess waste.

Experiment C

This experiment was conducted from 12 April 2002 to 11 June 2002 in order to evaluate survival and growth of prawn in culture tanks that were cleaned regularly versus not cleaned for the duration of the trial. Six rectangular tanks (400L) 208.3 cm long, 7.2 cm wide and 53.3 cm deep were used for this experiment. The culture tanks had 32,044 cm^2 of surface area. Each tank contained a PVC standpipe 5.08 cm in diameter and 40.6 cm high connected to a 5.08-cm PVC pipe that directed excess water to a drain in the floor. The top of each standpipe was covered with a 0.5-mm metal mesh to prevent spillover of prawn. Each tank had 12.7 cm of free-board to help prevent prawn from jumping out. Each tank was supported by four legs 11.4 cm in diameter and 38.1 cm high.

Each tank contained one substrate frame. Substrate was positioned vertically in tanks that were siphoned to allow for ease of cleaning, and positioned horizontally in tanks that were not siphoned. The frames were 27 cm high, 165 cm long, and 28 cm wide. Each frame contained three 30.5 x 160-cm layers of mesh suspended 9 cm apart. The added substrate, measuring horizontal surfaces only, increased tank surface area to 46,454 cm^2 (45%).

Treatments were assigned in a random systematic format, with each treatment having three replications. Tanks were either siphoned every other day or not siphoned at all. Prawn were fed twice daily at 0830 and 1800 hours. Prawn were individually counted and stocked randomly in groups of 500 at a density of 5/L into each of the culture tanks. A total of 12,000 prawn with an average individual weight of 0.016 g were stocked into the six rectangular tanks.

Ammonia and pH levels were monitored weekly beginning on 24 April 2002 to ensure that the unsiphoned tanks were not experiencing deteriorations in water quality. Ammonia and pH were measured using a Hach DREL/5 kit (Hach Co., Loveland, Colorado) and a YSI Model 60 handheld pH and temperature system, respectively.

Pond Grow-Out Experiment

This experiment was conducted from 6 June 2002 to 25 September 2002 in order to evaluate survival and growth of prawn grown in ponds with two different heights (0.61 and 1.22 m) of substrate. Six rectangular 0.04-ha ponds 24.8 m long to water surface, 16.5 m wide to water surface, and averaging approximately 1.37 m deep were used for this experiment (Figure 2). Each pond had 0.6 m of freeboard. The ponds were built on a slope in groups of two approximately 7.3 m apart and 18.3 m between pairs. Ponds 1 and 2 were at the highest elevation and Ponds 5 and 6 at the lowest elevation. A diversion ditch 0.3-m deep was built around each pond to help keep rain and runoff from entering the ponds. The ponds had a 2:1 inside slope, 3:1 outside slope, and a 1% bottom slope except leading into the catch basin where it was 2:1. Each pond had a catch basin 6.1 x 6.1 m with a PVC standpipe 1.82 m high and 20.3 cm in diameter connected to a 20.3-cm diameter PVC elbow located in the center of each. The PVC elbow directed water and prawn, during harvest, to an outlet located 4.6 m downslope. A propeller-type aerator (Kasco 110 volt) was used to aerate each pond 24 hours a day.

A soil test was performed on each experimental pond to determine pH prior to filling with water. Due to a leak the previous year, soda ash was used to seal pond 2. A 0.62-ha watershed pond was used to distribute water by gravity flow to each experimental pond. Water was routed through 10.2-cm diameter PVC pipes to the six ponds. Prior to filling the experimental ponds, the reservoir pond was treated with rotenone to kill any aquatic life; experimental ponds were subsequently filled with water four weeks prior to stocking. At that time a one-half bale of alfalfa hay and 4,536 g of cottonseed meal, and 363 g of inorganic fertilizer (19-19-19) were added to each pond and to the watershed

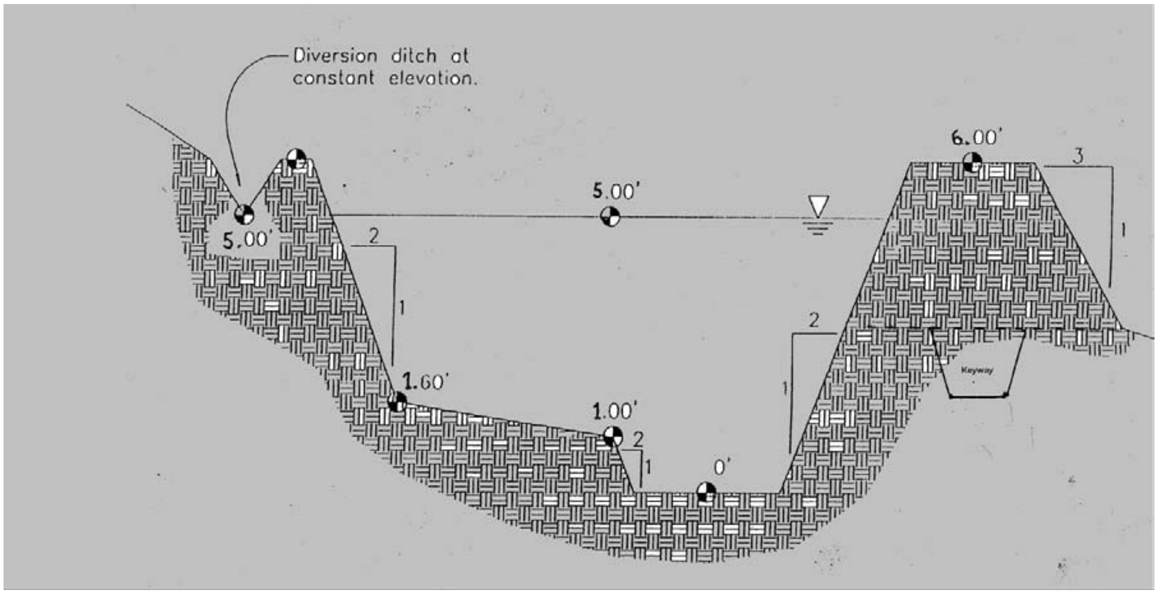

Figure 2. Schematic representation of pond layout.

pond for fertilization. The inorganic fertilizer was also added to each pond, at the same rate, two weeks after stocking.

Treatments were assigned in a systematic random format, with each treatment having three replications. Substrate was installed into each of the ponds prior to filling with water. Nine 15-m long rows of polyethylene fencing with a mesh size of 51 x 76-mm were suspended vertically in each of the six ponds. In three ponds, 0.61 m-high fencing was used to increase available surface area by 20%, and 1.22 m high fencing was used in the other three ponds to increase available surface area by 40% (Figure 3). The bottom edge of each fence was in contact with the pond bottom.

Figure 3. Height of substrate in ponds: 0.61 m (left), 1.22 m (right)

Stocking and Pond Management

Prawn from Experiment A were used for this study. Prawn were shipped via ground transport at a density of 5/L in two 400-L hauling tanks. A total of 14,400 prawn averaging 0.14 g were individually counted and stocked at a density of 53,305/ha into each pond. In all ponds, dissolved oxygen (DO) and temperature were monitored weekly. Prawn were fed a commercially pelleted sinking catfish feed (Tennessee Farmer's Co-op) containing 32% protein, 5% fat, <7% fiber, and <10% ash. Feed was stored on a wooden pallet and kept cool in a metal building with a concrete floor. The initial feeding rates were based on Craig Upstrom's growth curve (Aquaculture of Texas, Weatherford, Texas). Feed amounts were increased bi-weekly according to field observations throughout the duration of the study and estimated mortality. Mortality was based on Upstrom's growth curve and set at 2% per month. Prawn were not fed for the first 12 days of the experiment. During this time, prawn were able to feed upon macro-invertebrates inhabiting the pond. For the entire study period, prawn were fed half of the daily amount twice daily except for weekends when they were fed once per day.

Harvest

Prawn were harvested after 106 days on 25 September 2002. Water clarity was recorded for each pond prior to draining. All ponds were completely drained by turning down the stand pipes, beginning with Pond 5. Prawn were collected outside each pond from the outlet pipe with a 0.6-cm mesh seine. Prawn that remained on the pond bottom were collected manually. Prawn were rinsed with water to remove mud and debris and then purged in clean water for approximately 30 minutes (Figure 4). After being purged in water, the prawn were chilled in an ice bath for 15 minutes to quickly kill and preserve integrity of muscle tissue. Wooden purge boxes 0.6 m long, 0.6 m wide, and 0.46 m high with 0.2-mm hardware cloth on the bottom were used to rinse and chill the prawn in stock water tanks 2.4 m long and 0.9 m wide. After the ice bath, prawn were individually counted and weighed in batches on a digital Mettler scale (Accu-Weigh).

Statistical Analyses

Treatments were evaluated using the final mean survival numbers and final average individual weights of prawn. One way analyses of variance (ANOVA) were used to detect differences in survival and growth among treatments in experiments A, B, and C, and the pond grow-out experiment. A two-way ANOVA (2 x 2 factorial design)

Figure 4. Prawn being cleaned and chilled using a wooden purge box.

was used to detect differences in survival and growth of prawn stocked at 5 and 7.5/L and two levels of substrate in experiment A. Differences were considered significant at a $p < 0.05$ level. The SPSS Base 11.0 (2002) statistical analyses computer package was used for all tests.

CHAPTER IV

RESULTS

Nursery Experiments

Experiment A

Water quality parameters including temperature, dissolved oxygen, pH, salinity, carbon dioxide, ammonia, and nitrite were recorded upon arrival for each bag of prawn that had been shipped. Temperatures ranged from 20.9-21.3°C, dissolved oxygen 201-263% saturation, pH 6.79-7.03, salinity 4.5-4.6 ppt, carbon dioxide 20-45 mg/L, ammonia 0.193-0.287 mg/L, and nitrite 0.057-0.082 mg/L. Survival for the transport was 84.5%. For analysis purposes, this experiment was divided into two concurrent studies.

The first study compared two amounts of substrate (50% (small) and 100% (large) increase in surface area) at two densities (5 and 7.5/L) in a two-way randomized complete block (RCB) design. A two-way ANOVA was run comparing survival and average individual weight after 30, 45, and 60 days (Figures 5, 6, 7, and 8). After 30 days, there were no significant differences found in survival (79 and 80% for 5 and 7.5/L, respectively) or weight (0.04 g) between the two densities. Similarly, there were no significant differences in survival (80 and 79% for small and large substrate amounts, respectively) or weight (0.04 g) between the small or large substrate amounts.

After 45 days, there were no significant differences in survival (76 and 77% for 5 and 7.5/L, respectively); however, prawn stocked at 5/L had a significantly lower weight (0.07 g) than prawn stocked at 7.5/L (0.08 g). There were no significant differences found in survival between the two substrate amounts (76%); however, prawn in tanks with the

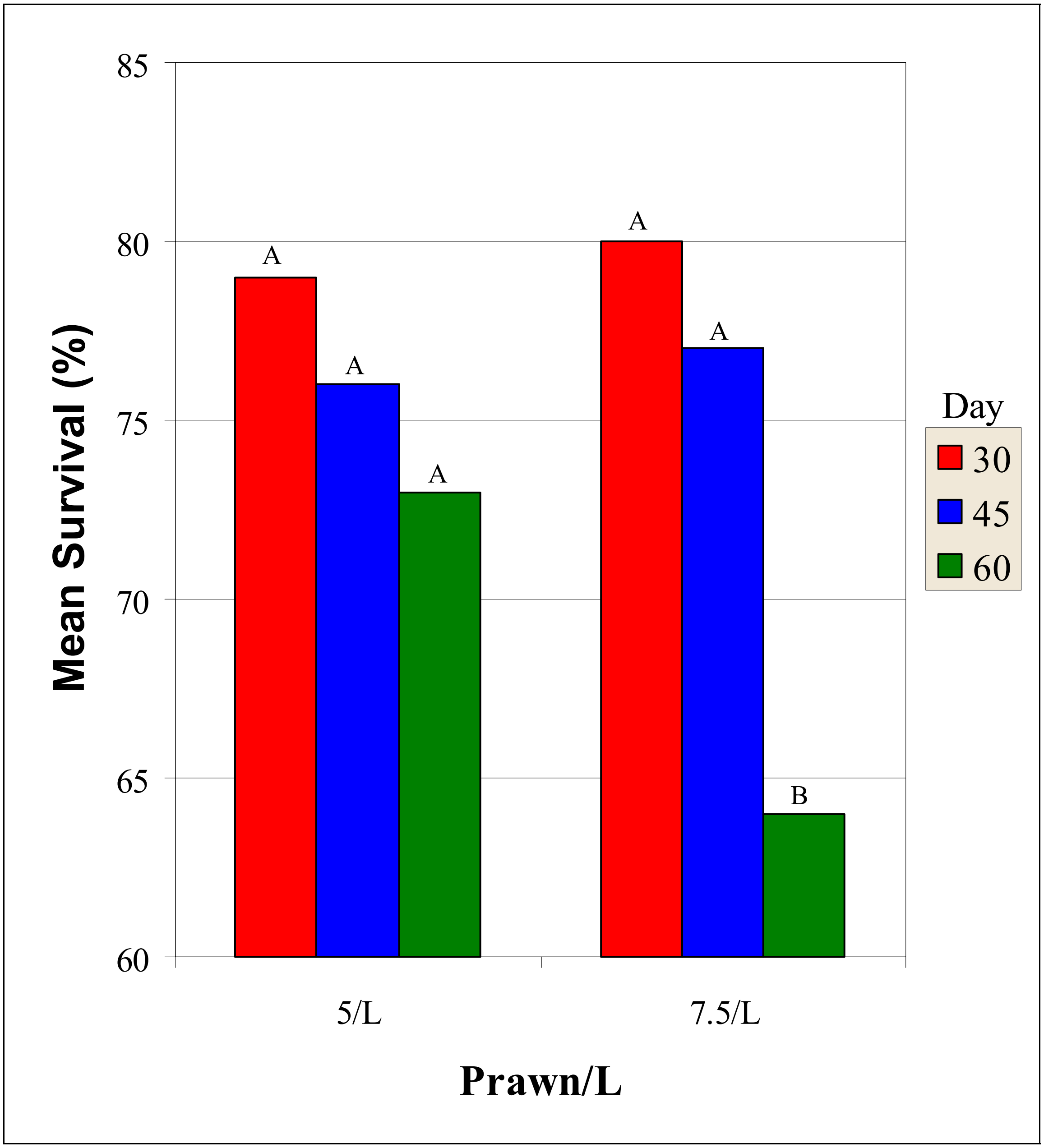

Figure 5. Mean survival (%) at 30, 45, and 60 days for prawn stocked at 5 and 7.5/L (significant differences in mean survival between the two densities on sampling days denoted as A vs. B).

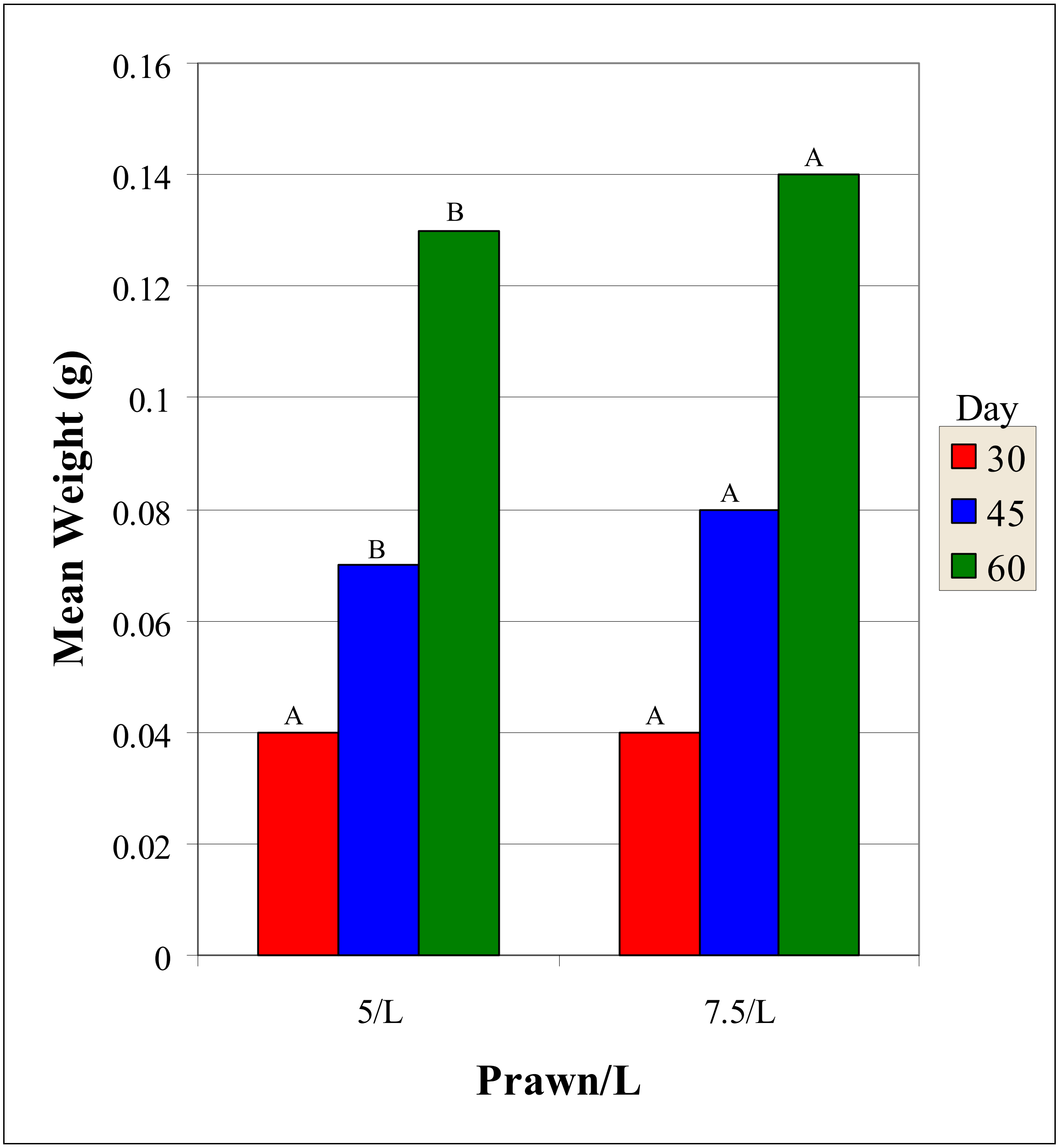

Figure 6. Mean Weight (g) at 30, 45, and 60 days for prawn stocked at 5 and 7.5/L (significant differences in mean weight between the two densities on sampling days denoted as A vs. B).

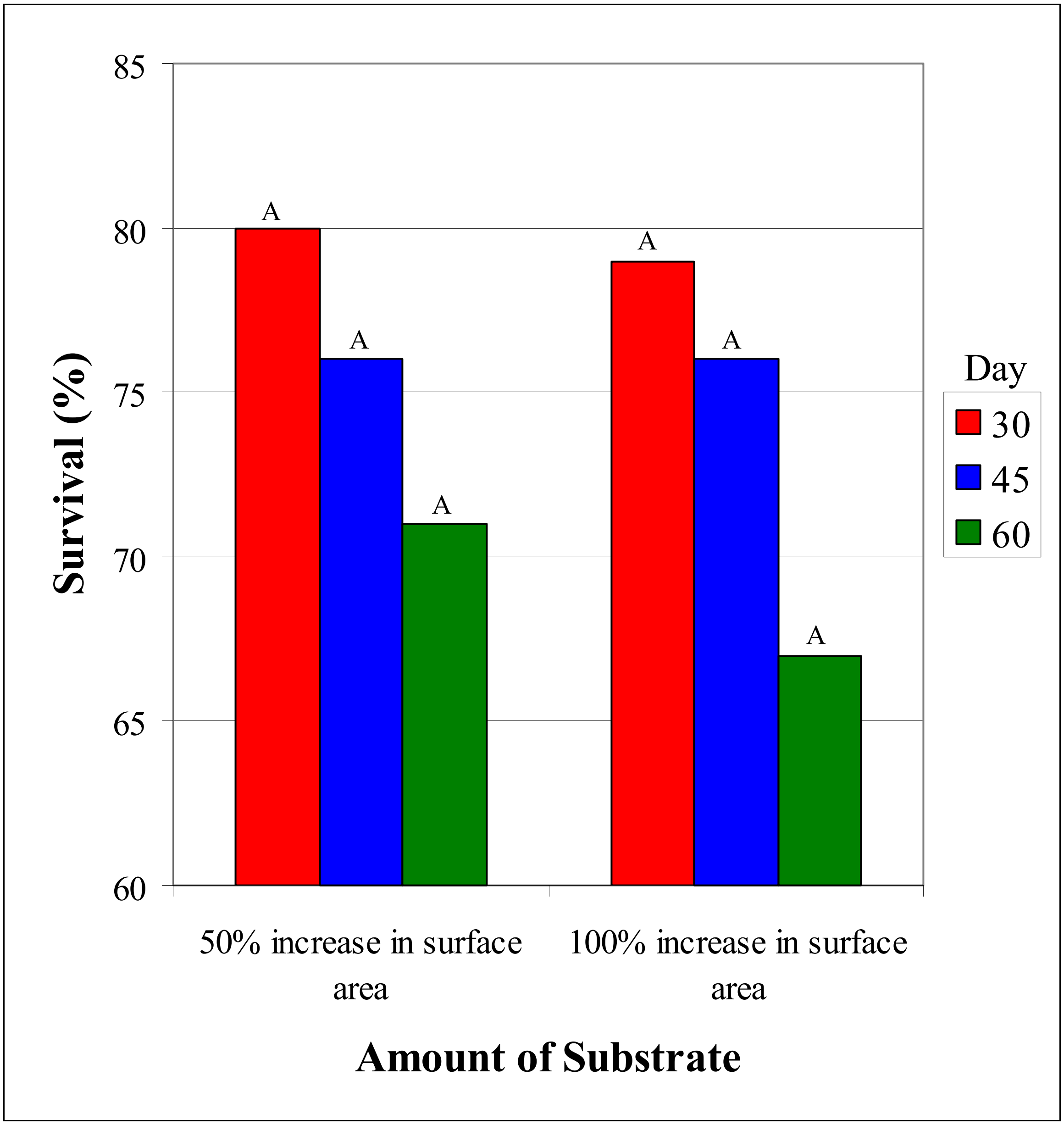

Figure 7. Mean survival (%) at 30, 45, and 60 days for prawn stocked with substrate equal to 50 or 100% increase in surface area (significant differences in mean survival between the two amounts of substrate on sampling days denoted as A vs. B).

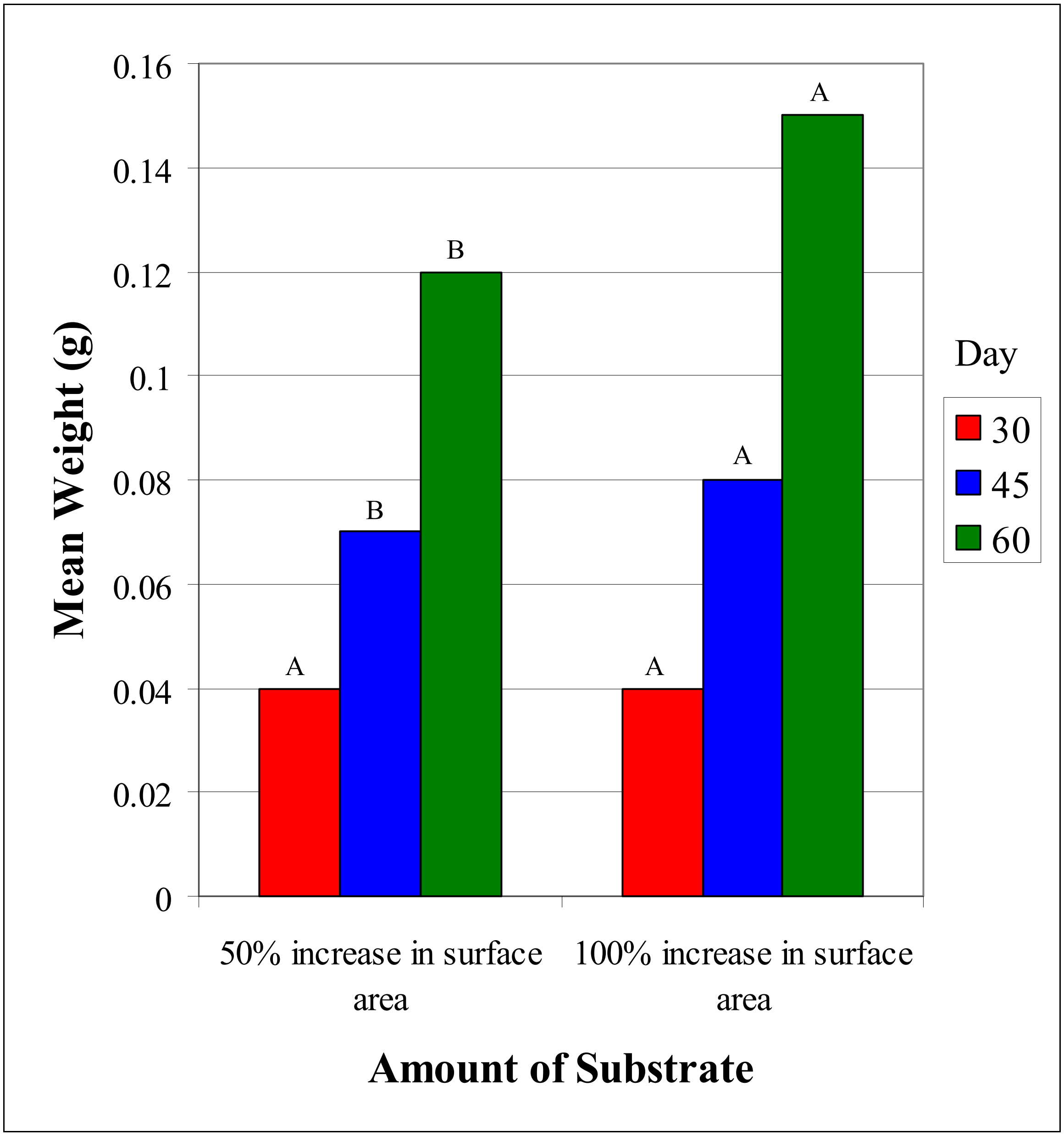

Figure 8. Mean weight (g) at 30, 45, and 60 days for prawn stocked with substrate equal to 50 or 100% increase in surface area (significant differences in mean weight between the two amounts of substrate on sampling days denoted as A vs. B).

small substrate had significantly lower weight (0.07 g) than prawn in tanks with large amount of substrate (0.08 g).

After 60 days, there was no statistically significant interaction between stocking density and amount of substrate for either survival or average weight. Prawn stocked at 5/L had a significantly higher survival (73 and 64% for 5 and 7.5/L), but lower average weight (0.13 vs. 0.14 g) than those stocked at 7.5/L. The large substrate amount did not significantly increase the survival of prawn (71% and 67% for small and large substrate amounts, respectively), but prawn in tanks with the large substrate amount were significantly larger (0.15 vs. 0.12 g) than those in tanks with the small substrate amount.

Percent mortality rates were 23, 24, and 28% at days 30, 45, and 60 for prawn stocked at a density of 5/L with small substrate. For prawn stocked at 5/L in tanks with large substrate, mortality rates were 19, 22, and 25% for days 30, 45, and 60, respectively. Prawn stocked at 7.5/L with small or large substrate amount had mortality rates of 19, 24, and 38%, and 20, 25, and 33% for days 30, 45, and 60, respectively. Prawn stocked at 5/L with small substrate grew from 0.04g at 30 days to 0.07g at 45 days, and to a final weight of 0.12 g at 60 days. The average weight of prawn stocked at 5/L with the large substrate amount was 0.04 g on day 30, 0.08 g on day 45, and 0.14 g on day 60. Average weights of prawn stocked at 7.5/L with the small substrate amount were 0.04, 0.08, and 0.13 g, and 0.04, 0.09, and 0.15 g for prawn stocked in tanks with the large substrate amount at days 30,45, and 60, respectively. We concluded, from the above numbers, that mortality was highest between days 1 and 30 for all treatments. Greatest growth rates were observed between days 45 and 60.

The second study compared four different stocking densities (2.5, 5, 7.5, and 10/L) with the same amount of substrate (50% increase in surface area). A one-way ANOVA was run comparing survival and weight of prawn after 30, 45, and 60 days (Figures 9 and 10). After 30 days, prawn stocked at 2.5 and 5/L had significantly lower survival (76 and 77%) than prawn stocked at 7.5 and 10/L (81 and 84%), respectively. Prawn stocked at 2.5/L had a significantly lower average weight (0.03 g) than prawn stocked at 5, 7.5, or 10/L, which all had an average weight of 0.04 g.

Prawn stocked at 2.5/L had significantly lower survival (63%) than prawn stocked at 5 (75%), 7.5 (76%), or 10/L (77%), after 45 days. No significant differences in average individual weight were observed in prawn stocked at 7.5 or 10/L (0.08 g for both densities). Prawn stocked at 2.5/L had a significantly lower weight (0.05 g) than prawn stocked at 5/L (0.07 g).

After 60 days, there were no significant differences found in survival of prawn stocked at 5 (72%), 7.5 (62%) or 10/L (64%); however, prawn stocked at 2.5/L had significantly lower survival (55%) than prawn stocked at 5/L. No significant differences in average individual weight were found among prawn stocked at 5 (0.12 g), 7.5 (0.13 g), or 10/L (0.13 g). However, prawn stocked at 2.5/L had an average individual weight (0.08 g) that was significantly lower than prawn stocked at the three higher densities.

Survival gradually decreased over time. Overall, mortality was 45, 28, 38, and 36% for prawn stocked at 2.5, 5, 7.5, and 10/L, respectively. An average of 21% mortality occurred between days 1 and 30, 9% between days 30 and 45, and 10% between days 45 and 60. Overall, prawn grew an average of 0.03 g between days 1 and 30, gained 0.03 g between days 30 to 45, and finally, between days 45 and 60, had an

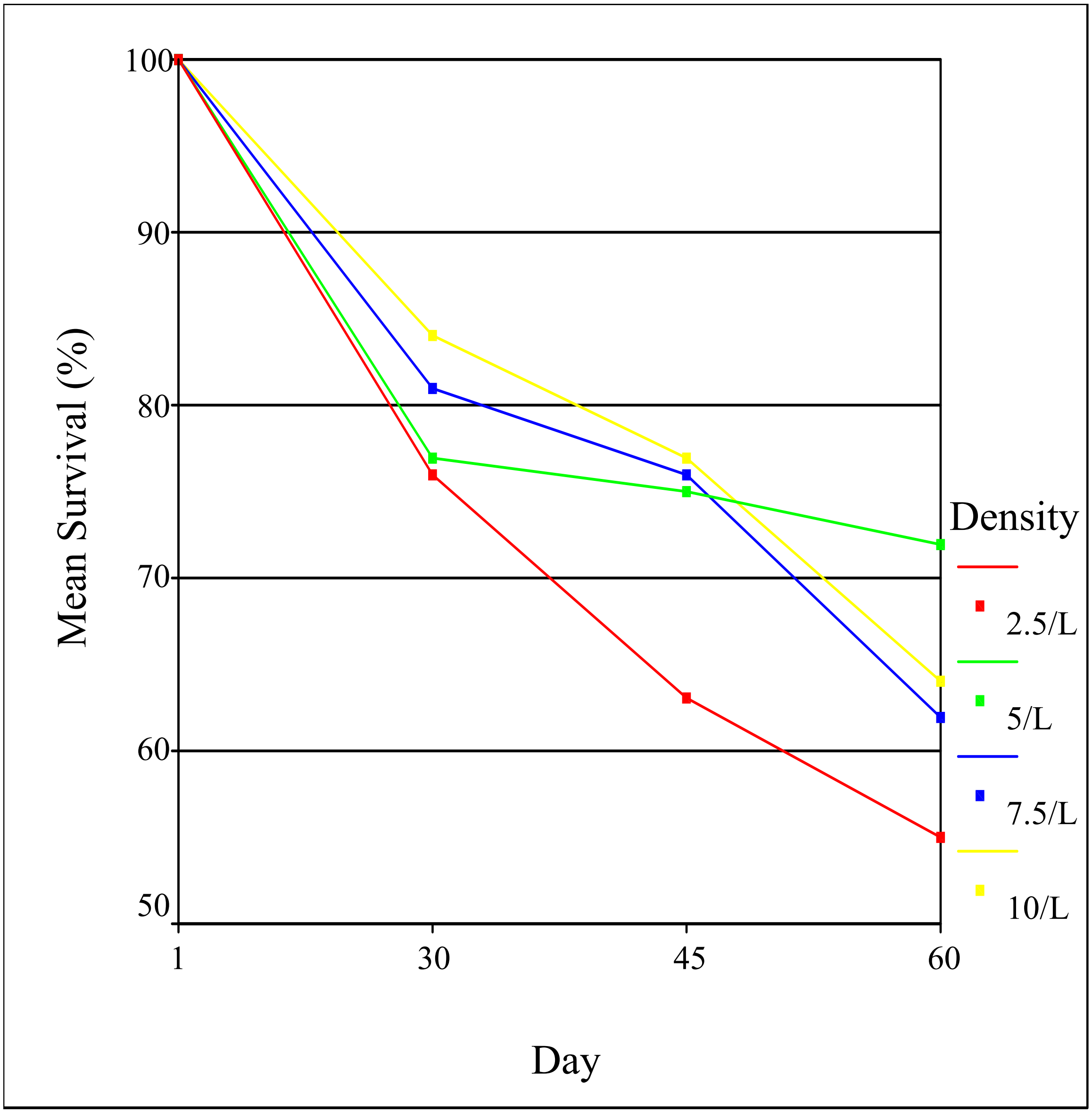

Figure 9. Mean survival (%) at 30, 45, and 60 days for prawn stocked at 2.5, 5, 7.5, and 10/L.

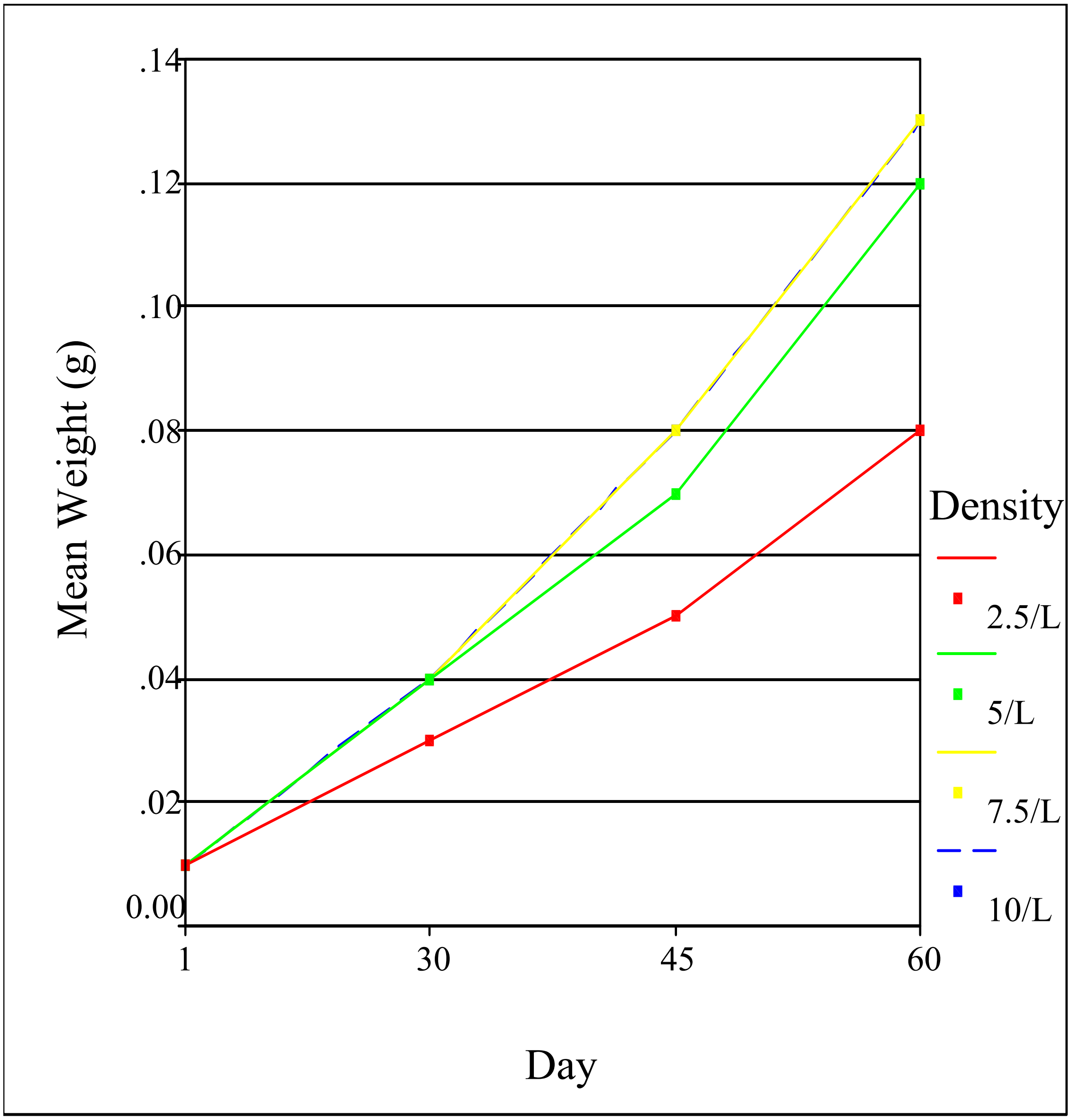

Figure 10. Mean weight (g) at 30, 45, and 60 days for prawn stocked at 2.5, 5, 7.5, and 10/L.

average individual weight gain of 0.05 g. From these numbers we conclude that prawn growth was greatest between days 45 and 60.

Experiment B

In the frequency of feeding experiment, no significant differences in survival of prawn fed once daily versus twice daily were found after 60 days. Survival numbers were 1471, 1367, and 1247 for prawn fed once daily and 731, 1262, and 1308 for prawn fed twice daily, respectively. These figures represent total mean survival rates of 66% for prawn fed once daily and 55% for prawn fed twice daily.

Similarly, mean weights were not significantly different between prawn fed once daily versus twice daily after 60 days. Average individual weight of prawn fed once daily was 0.12, 0.13, and 0.13 g. Prawn fed twice daily had average individual weights of 0.14, 0.12, and 0.13g. Mean weight of prawn in both treatments was 0.13 g.

Using SPSS (2002) (Table 1), a one-way ANOVA was run comparing final survival and final weight of prawn between the two treatments. The ANOVA yielded significance values of 0.250 and 0.643 for survival and weight, respectively. Therefore, neither treatment was significant in producing higher survival numbers or weight gain over the other. Overall, an average mortality of 32% was recorded for prawn fed once daily. Prawn fed twice daily had an average mortality of 45%. In both treatments, prawn gained an average of 0.11 grams throughout the duration of the study.

Table 1. One-way ANOVA comparing survival and weight gain of prawn as influenced by frequency of feeding.

		Sum of Squares	df	Mean Square	F	Sig.
Survival	Between Groups	253.500	1	253.500	1.811	.250
	Within Groups	560.000	4	140.000		
	Total	813.500	5			
Weight	Between Groups	.000	1	.000	.250	.643
	Within Groups	.000	4	.000		
	Total	.000	5			

Experiment C

In the waste removal experiment, no significant differences were found after 60 days in survival of prawn in tanks where waste and uneaten feed were removed versus prawn in tanks where waste was allowed to accumulate. Survival numbers were 1098, 1051, and 1269 for prawn in tanks that were siphoned and 1189, 1501, and 1311 for prawn in tanks that were not siphoned, respectively. These figures represent total mean survival rates of 57% for prawn in tanks where waste was removed and 67% for prawn in tanks where waste was allowed to accumulate.

Similarly, mean weight of prawn was not significantly different between the two treatments after 60 days. Average individual weight of prawn in tanks where waste was removed were 0.12, 0.11, and 0.09 g and 0.15, 0.12, and 0.14 g for prawn in tanks where waste was allowed to accumulate, respectively. Mean weight of prawn in tanks which were siphoned was 0.11 g and 0.14 g for prawn in tanks that were never siphoned.

A one-way ANOVA was run using SPSS (2002) (Table 2). After 60 days, there were no significant differences in survival or average individual weight found between the two treatments. Overall, an average mortality of 43% was seen for prawn in tanks that were siphoned. Prawn in tanks that were not siphoned experienced 33% mortality. Prawn gained an average of 0.09 g in tanks that were siphoned and 0.12 g in tanks that were not siphoned.

Total ammonia ranged from 0.004 to 0.138 mg/L in tanks where waste was removed and 0.022 to 0.159 in tanks where waste and uneaten feed were allowed to accumulate, respectively (Table 3). Unionized ammonia ranged from 0.12 to 1.57 mg/L in tanks that were siphoned and 0.11 to 1.6 mg/L in tanks that were not siphoned. The range of pH was 6.26 to 7.43 for tanks that were siphoned and 6.24 to 7.44 for tanks that were siphoned. Temperatures ranged from 22.7 to 27.0°C throughout the duration of the experiment.

Table 2. One-way ANOVA comparing survival and weight gain of prawn as influenced by waste removal.

		Sum of Squares	df	Mean Square	F	Sig.
Survival	Between Groups	150.000	1	150.000	3.529	.133
	Within Groups	170.000	4	42.500		
	Total	320.000	5			
Weight	Between Groups	.001	1	.001	5.786	.074
	Within Groups	.001	4	.000		
	Total	.002	5			

Table 3. The minimum (Min), maximum (Max), and mean total ammonia and unionized ammonia levels and minimum (Min) and maximum (Max) pH levels for all replicates of each treatment.

		Total Ammonia (mg/L)			Unionized Ammonia (mg/L)			pH	
Tank	Treatment	Min	Max	Mean	Min	Max	Mean	Min	Max
1	Siphoned	0.009	0.116	0.062	0.119	1.199	0.559	6.26	7.30
2	Not Siphoned	0.022	0.159	0.096	0.112	1.118	0.554	6.24	7.26
3	Siphoned	0.004	0.125	0.063	0.365	1.325	0.775	6.77	7.35
4	Not Siphoned	0.034	0.138	0.081	0.591	1.444	0.957	6.99	7.40
5	Siphoned	0.022	0.138	0.075	0.699	1.567	0.996	7.06	7.43
6	Not Siphoned	0.022	0.142	0.095	0.707	1.603	0.978	7.10	7.44

Pond Grow-Out Experiment

A soil test was performed on each experimental pond to determine pH prior to filling with water (Table 4). Pond 2 had a significantly higher pH (9.4) than the five other ponds. Throughout the experiment, dissolved oxygen measurements were at least 2 ppm and the water temperature was never below 20°C, proving suitable for growth (New and Valenti 2000). Water clarity was also recorded for each pond at harvest.

After 106 days, no significant differences were detected in survival between ponds using the two fencing heights. Survival numbers were 932 (39%), 2183 (91%), and 1915 (80%) for ponds with 0.61-m high substrate, and 2293 (96%), 1790 (75%), and 2044 (85%) for ponds with 1.22-m high substrate. The above figures represent total mean survival rates of 70 and 85% for ponds with 0.61 and 1.22-m high substrate, respectively.

Similarly, no significant differences were found in yield, average individual weight, or feed conversion between the two treatments. Ponds with 0.61-m high substrate had yields of 33.83, 67.22, and 56.25 kg. Prawn in this treatment had average individual weights of 36.33, 30.82, and 29.39 g. Yields in ponds with 1.22-m high

Table 4. pH and water clarity measurements for experimental ponds.

Pond	pH	Water Clarity
1	5.9	clear
2	9.4	very muddy
3	5.5	cloudy
4	5.5	cloudy
5	5.3	cloudy
6	5.2	muddy

substrate were 68.33, 54.36, and 60.39 kg and the average individual weight of prawn in these ponds was 29.82, 30.39, and 29.57 g. Over the duration of the experiment, each of the six ponds was fed 112.4 kg of feed.

Using SPSS (2002), a one-way ANOVA (Table 5) was run to compare survival, yield, and average individual weight of prawn between the two treatments. The ANOVA yielded significance values of 0.420, 0.464, and 0.350 for survival, yield, and weight, respectively. Therefore, neither treatment was significant in producing higher survival, yield, or weight over the other.

Overall, ponds with 0.61-m high substrate had an average mortality rate of 30%, a yield of 1296 kg/ha, and an average individual weight of 32.2 g. Ponds with 1.22-m high substrate had an average mortality rate of 15%, a yield of 1508 kg/ha, and an average individual weight of 29.9 g. Ponds with 0.61 and 1.22-m high substrate had feed conversion ratio of 2.14 and 1.84, respectively.

Table 5. One-way ANOVA comparing survival, yield, and weight of prawn as influenced by substrate height.

		Sum of Squares	df	Mean Square	F	Sig.
Survival	Between Groups	348.234	1	348.234	.807	.420
	Within Groups	1725.921	4	431.480		
	Total	2074.155	5			
Yield	Between Groups	538.654	1	538.654	.655	.464
	Within Groups	3291.580	4	822.895		
	Total	3830.234	5			
Weight	Between Groups	7.616	1	7.616	1.120	.350
	Within Groups	27.209	4	6.802		
	Total	34.826	5			

CHAPTER V

DISCUSSION

Previous studies have evaluated the response of juvenile prawn to increases in density and surface area. However, these studies have been difficult to compare due to variations in culture system design, added substrate, duration of culture, stocking size, and density. In the present nursery experiments, stocking density was expressed as number of prawn per volume of water because the substrate allows prawn to utilize the entire water column.

In a study conducted by Kneale and Wang (1979), survival was found to be inversely proportional to initial stocking density. Survival was highest (84%) at a stocking density of 150/m^2 (0.54/L) and lowest (39%) at a density of 1,500/m^2 (5.4/L). Prawn stocked at a density equal to or less than 600/m^2 (2.18/L) had a mean final weight ranging from 0.8 to 1.0 g and 0.4 g for prawn reared at densities greater than 600/m^2. Smith et al. (1983) found no differences in survival of prawn reared at densities ranging from 1,194 to 1,663/m^2 (1.4 and 5.9/L); however, when initial stocking densities were increased to 2,511 and 6,376/m^2, (1.4 and 5.9/L), survival decreased to 78 and 55% after 8 weeks, respectively. Results of this study indicated that at a stocking density of 6,276/m^2, a culture period of 15 weeks was needed to produce prawn having a mean weight of 0.32 g. Molina (1990) reported prawn stocked at densities ranging from 7 to 11/L achieved mean final weights in the range of 0.33 to 0.42 g with survival ranging from 58 to 73%, after 7 weeks of culture. D'Abramo et al. (2002) recommended that prawn should be stocked at densities of 5 to 6/L in order to achieve the maximum growth and survival.

Kneale and Wang (1979) reported similar survival, but slightly better growth for prawn regardless of whether one or three habitat structures were provided per tank. Molina (1990) found no significant differences in growth when the number of habitats were increased from 2 to 4; however, at the highest initial stocking density, survival significantly increased with additional habitats.

In Experiment A of this study, prawn stocked at 5/L had higher survival, but lower average weight, than prawn stocked at 7.5/L. Also, additional substrate equal to 100% of the tank's surface area did not significantly increase survival over tanks with 50% added substrate, but prawn in tanks with substrate equal to an 100% increase in surface area did exhibit higher growth. In the second trial of Experiment A, initial densities ranged from 2.5 to 10/L. Prawn achieved a mean final weight ranging from 0.08 to 0.13 g with survival ranging from 55 to 72%. There were no significant differences in survival found between prawn stocked at 5, 7.5, or 10/L; however, prawn stocked at 2.5/L were significantly smaller than prawn stocked at 5/L. Prawn stocked at 2.5/L were significantly smaller than prawn stocked at the three higher densities. Feeding rates for the study were calculated by the number of prawn per tank. The researcher speculates that the quantity of feed distributed to prawn stocked at 2.5/L was not enough to cover the entire area of the culture tanks. This may have led to prawn exerting more energy foraging, and thus feeding less efficiently than prawn stocked at the higher densities. Findings from this study suggested that an initial stocking rate of 5/L may provide the best overall growth and survival. Prawn producers may be able to use a smaller size substrate to achieve similar survival rates while minimizing costs.

Costs associated with labor comprise a large portion of the operating expenses in prawn nurseries with feeding and waste removal being the two most intensive daily tasks. Most commercial producers feed prawn at least twice daily and it is generally thought that removal of uneaten feed and waste is a good management practice. Few studies have examined the effects of frequency of feeding or waste removal on prawn survival and growth. Heinen and Mensi (1991) found that prawn fed once daily exhibited better survival, final weight, and yield than prawn fed twice or three times daily. However, D'Abramo et al. (2002) recommended that the total daily ration should be divided into two or more separate feedings per day. Feeding more often should reduce losses of nutrients due to leaching; however, feeding once per day or allowing waste to accumulate may allow more time for microbial colonization, which may increase the nutrient suitability of the food. Feeding once per day may also allow the prawn to feed intermittently throughout the day.

The researcher also speculates that the siphoning procedure used to remove waste may cause stress sufficient to reduce growth. In the present study, there were no significant differences in survival or weight between prawn fed once versus twice daily. Similarly, no significant differences were found in survival or weight of prawn in tanks where waste was allowed to accumulate versus prawn in tanks where waste was periodically removed. However, prawn fed once daily had somewhat greater survival and prawn in tanks without waste removed had slightly increased growth.

In a supplemental study (data not included) conducted at the University of Tennessee, prawn averaging 0.007 g were reared in six circular and six round 400-L culture tanks. They were fed once daily and waste was allowed to accumulate for 45 days. This study resulted in an overall mean survival of 76% and a mean final individual weight of 0.07 g. This study further supported the two previous studies in suggesting that producers can save time and money by feeding once per day and not cleaning. The above studies were conducted in a flow-through aquaculture system which limited deterioration in water quality. Throughout the duration of the study, ammonia concentrations never reached levels that would be either stressful or lethal to the prawn. Unionized ammonia (NH_3) increases with pH and temperature and has a higher toxicity than ionized ammonia (NH_4). It is recommended that unionized ammonia levels be maintained below 1.8 mg/L (New and Valenti 2000).

Prawn in all of the nursery studies exhibited poor growth in comparison with previous studies. Prawn stocked in the nursery experiments had initial mean weights ranging from 0.010 to 0.016 g and final mean weights ranging from 0.08 to 0.14 g. Smith et al. (1983) suggested that postlarvae with an initial mean weight of 0.01 g needed a culture period of 9.5 to 11 weeks to obtain a mean weight ranging from 0.20 to 0.44 g. D'Abramo et al.(2002) recommended that water temperatures should range between 25 and 28°C for nursery culture. Similarly, Kneale and Wang (1979) found that prawn reared at 24°C had an average final mean weight of 0.23 g and prawn reared at 28°C had an average final mean weight of 0.41 g. In the present study, problems associated with the reservoir heaters led to temperatures in the culture tanks falling below 25°C, which we believe caused the reduced growth. Also, the culture period lasted only 60 days. A longer culture duration may have led to increased growth in the prawn.

Freshwater very aggressive and cannibalistic when reared under crowded conditions, a situation which may have caused a significant portion of the mortalities in the current studies. Two methods have been suggested for solving the problem of aggressiveness of this species. The methods are genetic selection for non-aggressive individuals, or physiological manipulation to suppress or eliminate the aggressive behavior (Smith and Sandifer 1975). Individual segregation of the animals has also been suggested as an alternative; however, while this may be technically feasible, it is economically prohibitive.

Previous studies have demonstrated a direct relationship between pond production intensity and amount of added substrate. Tidwell et al. (2001) reported production increased in a direct linear relationship with the amount of substrate added when prawn were stocked at 74,000/ha. Similarly, Tidwell et al. (2002) found that when prawn were stocked at 65,000/ha, yield was significantly greater in ponds with a 100% increase in surface area compared to ponds with a 50% increase in surface area. In the present study, no differences were detected in survival, yield, mean individual weight, or feed conversion between ponds using the two fencing heights. A soil test taken prior to stocking revealed that Pond 2 had a significantly higher pH (9.4) than the other ponds, which may have caused the high mortality (61%) in this pond. The high pH is this pond was likely caused by the addition of soda ash to seal the pond. New and Valenti (2000) reported that the ideal range for pH is 5.9-8.5. Although poor water visibility normally does not allow observation of the distribution of the prawn on the fencing, we speculate that prawn may primarily utilize the sections of fencing closest to the pond bottom. Farmers may be able to obtain similar results by using the shorter fencing height while minimizing costs. In this study, however, prawn were stocked 53,304/ha, a density which may be to low too demonstrate any differences between the two heights of substrate.

The process of sampling may have decreased survival and growth rates. We have no way to gauge the effects of sampling on survival and growth. Sampling may raise stress levels directly by exposing the prawn to air or indirectly by increasing the frequency of behavioral interactions. Aquaculture research practices are often more invasive than those used by commercial growers, with experimental animals being subjected to repeated handling and movement. The prawn industry has continued to grow over the last three years in Tennessee. Current and future studies will continue to explore ways to increase overall production while minimizing costs. Continued research into marketing strategies will also be vital to the success of the prawn industry.

CHAPTER VI

SUMMARY

Three nursery experiments were performed to evaluate the effects of stocking density, amount of added substrate, feeding frequency, and waste removal on the survival and growth of juvenile prawn. One pond experiment was conducted to examine the effects of substrate height on production. The major findings were:

Nursery Experiments

Experiment A

1. In the density and substrate experiment prawn were stocked at four different densities and two amounts of substrate. In the first part of the experiment, prawn were stocked at 5 and 7.5/L with layers of mesh added to equal 50 or 100% increase in surface area. Prawn stocked at 5/L had significantly higher survival (73 vs. 64%), but lower average weight (0.13 vs. 0.14 g) than those stocked at 7.5/L. Substrate equal to 100% of the tank's surface area did not significantly increase survival (71 vs. 67%), but did produce larger prawn (0.15 vs. 0.12 g) than substrate equal to 50% of the tank's surface area.
2. In the second part of the study, prawn were stocked at either 2.5, 5, 7.5, or 10/L with substrate equal to 50% of the tank's surface area. There were no differences found in survival of prawn stocked at 5 (72%), 7.5 (62%), or 10/L (64%); however, prawn stocked at 2.5/L had significantly lower survival (55%) than prawn stocked at 5/L. Prawn stocked at 2.5/L had an average

individual weight (0.08 g) that was significantly less than prawn stocked at 5 (0.12 g), 7.5 (0.13 g), or 10/L (0.13 g).

Experiment B

1. In the frequency of feeding experiment, survival and growth of prawn fed once a day were compared to prawn fed twice a day. There were no significant differences in survival between prawn fed once per day (66%) versus prawn fed twice per day (55%).
2. In the same experiment, the average individual weight for prawn fed once per day was 0.13 g. For prawn fed twice per day, the average individual weight was also 0.13 g, indicating that there were no significant differences in weight observed between the two treatments.

Experiment C

1. In the waste removal experiment, three tanks were siphoned three times weekly to remove uneaten feed; the three other tanks were never siphoned and uneaten feed and waste were allowed to accumulate. Survival was 57% for prawn in tanks where waste was removed, compared to 67% for prawn in tanks where waste was allowed to accumulate, indicating no significant differences in survival.
2. In the same experiment, final average weight for prawn in tanks where waste was removed was 0.11 g, compared to 0.14 g for prawn in tanks where waste was allowed to accumulate. No statistically significant differences in weight were observed between the two treatments.

Pond Grow-Out Experiment

1. In the pond experiment, three ponds had 1.22-m high fencing and three had 0.61-m high fencing, increasing surface area by 20 and 40%, respectively. No significant differences were found in survival. Survival was 70% for prawn in ponds with 0.61-m fencing, and 85% for prawn in ponds with 1.22-m high fencing.
2. Similarly, no significant differences were found in mean individual weight (32.2 vs. 29.9 g) or feed conversion (2.14 vs. 1.84) for ponds with 0.61 versus 1.22-m high substrate, respectively.

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